Molecular cloning, gene expression profiling and in silico sequence analysis of vitamin E biosynthetic genes from the oil palm

ABSTRACT

Homogentisate geranylgeranyl transferase (HGGT) and homogentisate phytyltransferase (HPT) are the two key enzymes involved in condensation of homogentisic acid (HGA) with a prenyldiphosphate to produce tocotrienols and tocopherols in plants, respectively. The partial cDNAs encoding HGGT and HPT enzymes were successfully isolated from the two oil palm species, Elaeis guineensis and Elaeis oleifera by PCR amplification using degenerate primers. Subsequently, full length cDNA sequences were completed by rapid amplification of cDNA ends (RACE) and further annotated using various bioinformatics tools. The analysis revealed the presence of an UbiA prenyltransferase conserved domain in all four deduced amino acid sequences and suggested that oil palm HGGT and HPT are more evolutionarily related to their counterparts from other monocot plant species. Quantitative gene expression analysis was carried out to elucidate the transcript profiles of the oil palm HGGT and HPT in different oil palm tissues and at different developmental stages of the mesocarp. The HPT was constitutively expressed in all analyzed tissues except in 15 w.a.a kernel whereas oil palm HGGT showed preferential expression in mesocarp and kernel tissues. However, HPT was highly expressed at the fruit ripening stage of 17 w.a.a mesocarp when active oil deposition occurs. Genome-walking PCR successfully amplified the promoter regions of HGGT and HPT from E. guineensis. Computational analysis using PlantCare and PLACE databases revealed several cis-regulatory elements including phytohormone-responsive, light-responsive and abiotic factor-responsive elements which may be involved in coordinating expression of both genes. Taken together, this study provides useful information about important features of the cDNA and promoter sequences as well as an insight into the transcriptional regulation of these key vitamin E genes for future genetic improvement efforts.

Keyword: Oil palm; Homogentisate phytyltransferase (HPT); Homogentisate geranylgeranyl transferase (HGGT); Degenerate primer; qPCR; Promoter analysis